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## Research report

## Remote long-term registrations of sleep-wake rhythms, core body temperature and activity in marmoset monkeys

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## HIGHLIGHTS

- ▶ The NeuroLogger® enables long-term recordings of sleep and activity in marmosets.
- ▶ The Remo200 system allowed for long-term measurement of core body temperature.
- ▶ Methods allow simultaneous registration of unrestrained pair-housed marmosets.
- ▶ Sleep deprivation leads to a homeostatic sleep rebound in marmosets.
- ▶ The marmoset is an interesting model for sleep and circadian research.

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## ABSTRACT

Initial studies in the day active marmoset monkey (*Callithrix jacchus*) indicate that the sleep-wake cycle of these non-human primates resembles that of humans and therefore conceivably represent an appropriate model for human sleep. The methods currently employed for sleep studies in marmosets are limited. The objective of this study was to employ and validate the use of specific remote monitoring system technologies that enable accurate long-term recordings of sleep-wake rhythms and the closely related rhythms of core body temperature (CBT) and locomotor activity in unrestrained group-housed marmosets. Additionally, a pilot sleep deprivation (SD) study was performed to test the recording systems in an applied experimental setup.

Our results show that marmosets typically exhibit a monophasic sleep pattern with cyclical alternations between NREM and REM sleep. CBT displays a pronounced daily rhythm and locomotor activity is primarily restricted to the light phase. SD caused an immediate increase in NREM sleep time and EEG slow-wave activity as well as a delayed REM sleep rebound that did not fully compensate for REM sleep that had been lost during SD.

In conclusion, the combination of two innovative technical approaches allows for simultaneous measurements of CBT, sleep cycles and activity in multiple subjects. The employment of these systems represents a significant refinement in terms of animal welfare and will enable many future applications and longitudinal studies of circadian rhythms in marmosets.

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## 1. Introduction

Sleep is not simply a state of rest, but a dynamic, complex and regulated process [1,2]. Physiological sleep is characterized by two distinct stages, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, which alternate in an ultradian fashion.

It is generally accepted that sleep is homeostatically regulated and that the need for sleep builds up during wakefulness. Extended wakefulness or sleep deprivation (SD) leads to an increased sleep drive and subsequent compensatory sleep rebound. During sleep recovery, NREM sleep may display a compensatory increase not only in duration, but also in intensity. NREM sleep intensity is reflected in electroencephalogram (EEG) recordings by slow wave activity and a spectral power in the 1–4 Hz delta range. It is highest in the beginning of sleep and gradually declines during the sleep phase [3–5]. Slow wave activity is clearly related to the duration of prior wakefulness [6–9].

In contrast, it is believed that REM sleep recovers largely by an increase in duration [3,4,10,11]. However, in some studies,

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slight changes in REM sleep EEG activity following SD were also reported, which suggests that there might also be a change in quality [6,10,12,13]. REM sleep rebounds are less predictable and may only occur after longer SD. There are still unanswered questions as to how REM sleep rebounds are regulated and if they are related to the duration of prior waking or rather prior NREM sleep instead [14–17].

Besides homeostatic processes, the sleep–wake cycle is also subject to circadian regulation. The daily rhythm of sleep is under control of an endogenous clock or pacemaker in the suprachiasmatic nucleus of the hypothalamus [18–20]. The endogenously generated rhythm is entrained to the 24 h environment by light signals that function as external time cues [21].

Despite strong homeostatic and circadian regulation, the sleep–wake cycle is extremely vulnerable to perturbations by various internal and external factors. In fact, sleep disturbances are among the most frequently reported health problems [22,23]. Sleep disorders also typically occur in a variety of mental and neurological disorders, such as depression, anxiety disorders and dementia [24–26]. Appropriate animal models may be helpful in order to better understand the regulation of sleep and the mechanisms underlying sleep disorders. Currently, the most widely-used animal models in sleep research are rodents, particularly rats and mice. Unlike humans however, these rodents are nocturnal and exhibit polyphasic sleep–wake cycles. As such the day active marmoset monkey may represent a valuable and more translational animal model of sleep.

The marmoset monkey (*Callithrix jacchus*) is a small, day-active, non-human primate that is native to Brazil. Marmosets are not threatened or endangered in the wild and breed well in controlled laboratory conditions. They are social animals and can easily be housed in groups. Their small body size of around 25 cm in length and a weight of 300–450 g enable the application of research tools originally developed for laboratory rodents such as rats [27–30].

Experiments designed to characterize diurnal rhythms of behavioral activities and physiological functions have shown that marmosets are excellent non-human primate models for basic research in chronobiology [31–35]. In their natural habitat, as well as under artificial and controlled lighting conditions in the laboratory, marmosets restrict their behavioral activities, such as locomotion, entirely to the light phase [32,36,37].

While the basic characteristics of circadian organization are fairly well described in marmosets, few studies were specifically aimed at sleep. Initial studies suggested that marmosets have a monophasic sleep period with a cyclic structure alternating between NREM and REM sleep phases throughout the night. This might indicate that the sleep–wake cycle of marmoset monkeys is similar to that of humans and therefore supports the use of marmosets as an appropriate model for human sleep [38]. However, the approaches and methods used for sleep studies in marmosets so far are limited and have not yet provided a complete picture of sleep-patterns and sleep homeostasis. Most previous studies consisted of telemetric recordings of EEG combined with video registrations [38–41]. These telemetric recordings depend on close proximity between the animal equipped with a transmitter and receivers near the sleeping box. Hence, since marmosets spend much of their time away from the sleeping box during the daytime, full 24 h recordings of sleep and wakefulness have not been performed. Importantly, a recent study in tree shrews showed that strictly day-active mammals may still show considerable amounts of sleep during the daytime hours, even when they are away from the sleeping box [42]. In addition, most previous studies in marmosets only assessed baseline patterns of night-time sleep [38,40]. In some cases, they also investigated how these patterns were affected by certain treatments like administration of organophosphate [39] or multiple vaccines [41]. A formal assessment of sleep

homeostasis, i.e., the response to sleep deprivation, has not yet been performed.

The overall objective of this study was to employ and validate the use of specific remote monitoring system technologies that enable accurate long-term recordings of sleep–wake rhythms and the closely related rhythms of core body temperature and locomotor activity in unrestrained single- and group-housed marmoset monkeys. We applied this technology to assess full 24 h sleep–wake patterns under baseline conditions and the homeostatic response to sleep deprivation.

## 2. Materials and methods

All experiments were performed in accordance with the European Communities Council Directive 86/609/EEC and the German Animal Welfare Act and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety, Germany.

### 2.1. Animals

Adult common marmoset monkeys (ages 4–10 years) were obtained from the breeding-colony of the German Primate Center (Göttingen, Germany). The animals were housed individually or in mixed-sex pairs (as required by the experimental setup) in a temperature- ( $25 \pm 1^\circ\text{C}$ ) and humidity-controlled ( $65 \pm 5\%$ ) facility. Illumination was provided by artificial lighting on our standard light cycle, consisting of a 12.5 h light phase with dusk and dawn effects for the first and last 30 min and a 11.5 h dark phase. Light levels measured in the middle of the cage were 600–650 Lux during the day and 350–400 Lux during dim light phases. Each cage ( $50 \times 98 \times 70$  cm, Ebeco, Castrop-Rauxel, Germany) was furnished with wooden branches and shelves, and contained either a metal ( $20 \times 20 \times 30$  cm, entrance  $15 \times 18$  cm) or a wooden ( $35.5 \times 26 \times 17$  cm) sleeping box. The animals were fed ad libitum with a pelleted marmoset diet (ssniff Spezialdiäten, Soest, Germany). In addition, 20 g mash per animal was served in the morning and they received 30 g clean-cut fruits or vegetables mixed with noodles or rice in the afternoon. Water was always available.

A total of 4 common marmosets (2 males and 2 females) were investigated in 2 independent experiments (long-term recording experiment and sleep deprivation experiment). We employed a minimal number of animals in this study since the primary goal was to establish the NeuroLogger® and Remo systems in the marmoset and to test their usability.

### 2.2. Experimental design

#### 2.2.1. Long-term recording experiment

A mixed-sex pair of marmosets (intact female: aged 8 years, bodyweight 440–460 g and castrated male: aged 10 years, bodyweight 430–450 g) was instrumented for the recording of sleep parameters, core body temperature and locomotor activity. The recording of core body temperature was performed continuously, while sleep and activity were recorded in specific time windows 2, 3, 6 and 9 weeks after implantation (see Fig. 1). Each recording period consisted of 7 days continuous NeuroLogger®-recordings with daily exchange of the recording device at different times of the day.

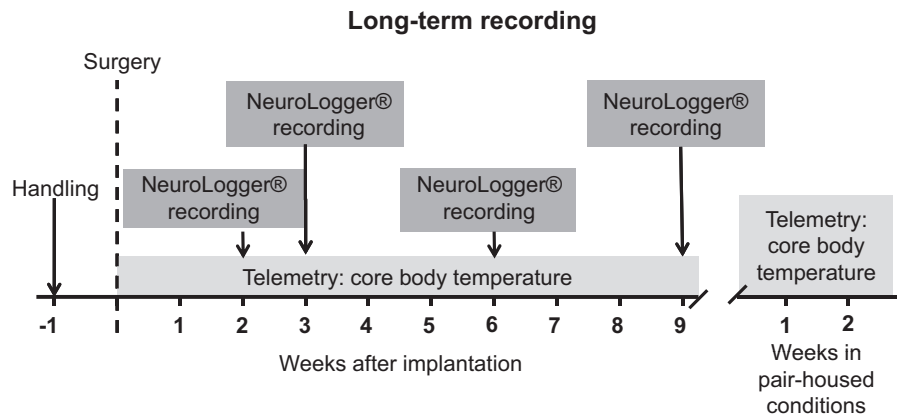
For initial sleep and activity recordings, the animals were housed singly in neighboring cages that enabled visual and acoustic contact between the animals. Subsequently, the animals were pair-housed to assess the physical stability of the utilized recording devices (female: 12th week after implantation; male: 10th week after implantation).

#### 2.2.2. Sleep deprivation experiment

A separate mixed-sex pair of marmosets (intact female: aged 4 years, bodyweight 480–500 g, last birth 1.5 years prior to experiment and intact male: aged 5 years, bodyweight 460–480 g) was instrumented for the recording of sleep parameters and activity. Core body temperature was not recorded in this experiment. Following surgical instrumentation, the animals were kept in individual cages for one week to allow for recovery. They were returned to the pair-housed condition after complete healing of surgical wounds. The animals were then allowed at least one additional week to recover before the start of the experiment. Baseline recordings in pair-housed conditions were followed by sleep deprivation (SD) for 6 h starting at dark onset. Each animal was subjected to two SD sessions separated by one week. Due to technical difficulties in the first SD session, only data from the second SD were analyzed. During SD, the sleep boxes were removed and the animals were kept awake by means of mild stimulation by an experimenter that was present in the room (tapping on the cage or gently touching the animal with a brush).

### 2.3. Recording devices

We utilized 2 newly developed recording devices for registrations performed in this study. The NeuroLogger® (Newbehavior, Zurich, Switzerland) was adapted for



**Fig. 1.** Timeline of the long-term recording experiment. The registration of core body temperature was performed continuously until the end of the experiment. Sleep and activity were recorded in four 7-day long periods (2, 3, 6 and 9 weeks after implantation).

use in freely moving marmoset monkeys to determine sleep parameters and locomotor activity via EEG, EMG and actimetry recordings. The recording of core body temperature was performed with Remo 200 radiotelemetry-transmitters (Remo Technologies Ltd., Salisbury, UK).

### 2.3.1. NeuroLogger®

The NeuroLogger® system, a datalogger system originally developed for use in small laboratory rodents, was used to record EEG and EMG activity as well as actimetry. Due to its small size ( $22 \times 15 \times 8$  mm) and weight of less than 2.5 g, this system is suitable for use in mice [43–45] and has also been used for recordings in flying pigeons [46,47] and sloths in their natural habitat [48].

Attached to a surgically implanted headmount, the NeuroLogger® enables wireless registration of up to four EEG/EMG channels with two references and actimetry via the included accelerometer. The recording frequency is programmable in the range of 100–500 Hz. Registered data is stored directly on the removable memory chip of the NeuroLogger® after amplification (input range  $\pm 500$   $\mu$ V) and bandpass-filtering (1–70 Hz, first order). Therefore, it is possible to make simultaneous recordings of multiple animals per cage/room.

To adapt the NeuroLogger® for use in marmoset monkeys, we developed a protective cover from carbon fiber reinforced plastic that was placed over the removable logger and fixed with screws to the implanted headmount (see Fig. 2). This design ensured a secure connection and protection of the device during recording.

### 2.3.2. Remo 200 transmitter

The recording of core body temperature (CBT) was performed with the Remo 200 radiotelemetry system. The transmitter ( $35 \times 14 \times 14$  mm; 11 g) was implanted in the abdominal cavity. The continuously recorded data were transmitted in 10 s-intervals to a receiver that was placed 10 cm above the animals' home cage. Besides the recorded data, each transmitter sends an individual identification number and therefore enables the recording of multiple animals simultaneously in the same experimental setting.

### 2.4. Implantation of telemetry transmitters and fixation of NeuroLogger® headmounts

All surgeries were performed under sterile conditions. General anesthesia was introduced with a mixture of ketamine (50 mg/ml), xylazine (50 mg/ml) and

atropine (0.1 mg/ml) at a dosage of 1 ml/kg bodyweight. After induction of anesthesia, each animal was shaved in the regio umbilicalis for implantation of the Remo 200 telemetry transmitters and in the regio occipitalis for fixation of the NeuroLogger® headmount. Then the animals were intubated with a polyethylene endotracheal tube (inner/outer diameters = 1.75/2.08 mm) and kept under inhalation anesthesia (0.5–2% isoflurane in 1:2 O<sub>2</sub>:N<sub>2</sub> overflow) with an animal respirator (Model Advanced, TSE, Bad Homburg, Germany), while body temperature was maintained at 38 °C using a heating pad (Horn, Gottmarding, Germany). Effective depth of anesthesia was confirmed repeatedly by checking for an absent pedal withdrawal reflex.

The animals were in the dorsal supine position for the implantation of telemetry transmitters. A 2 cm abdominal midline incision was made to open the peritoneal cavity. The sterile Remo200 transmitter was inserted and fixed to the abdominal wall with a 4–0 non-absorbable mersilene suture. The rectus abdominis muscle was closed with the same suture. Subsequently, the skin was closed with a 5–0 absorbable vicryl suture.

For the fixation of the NeuroLogger® headmount, the animals were then placed in sternal recumbency and the head was fixed in a stereotactic frame (David Kopf Instruments, Tujunga, CA, USA). The skin on top of the head was opened with a 3 cm midline incision to expose the skull. After cleaning and drying the surface of the skull with 3% hydrogen peroxide, four 1.0 mm holes were drilled through the skull bone over the frontal and parietal cortex using a micro drill (Stryker GmbH & Co. KG, Duisburg, Germany). Gold-plated brass screws (1.2 mm in diameter) were inserted into the holes and served as electrodes. The reference electrode was located on the right side approximately 8 mm anterior from bregma and 2 mm lateral to the midline. Two of the recording electrodes were also located on the right side, approximately 3 and 5 mm posterior to the reference. The third recording electrode was placed on the contralateral side over the parietal cortex, approximately 3 mm posterior from bregma and 2 mm lateral to the midline. Short silver-plated elastic cables were connected to the screws. To record EMG activity, two single-stranded stainless steel wires were implanted in the dorsal neck musculature and fixed with non-absorbable 4–0 Mersilene suture. All electrode leads were connected to a flat 7-pin 1.27 mm male pitch connector, which was fixed to the skull with dental acrylic (Paladur, Heraeus Kulzer, Hanau, Germany). Two screw nuts for the connection of the protective cover were anchored in the dental acrylic. The skin around the connector was closed using resorbable 5–0 vicryl sutures. A dummy with the same size and weight as a NeuroLogger® was connected to the pins and the protective cover was connected via screws over the dummy.

The animals were monitored until they awoke from anesthesia and were then returned to individual cages, identical to their homecages. Animals received intramuscular injections of Meloxicam (2.5 mg/kg bodyweight) approximately 20 min prior to and for 3 days following surgery as well as prophylactic antibiotics with amoxicillin (60 mg/kg) every 48 h for 5 days.

### 2.5. Recording and data processing

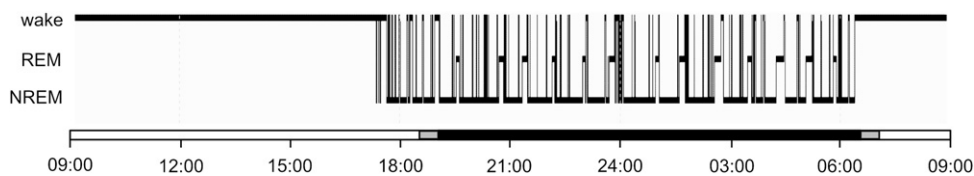
#### 2.5.1. Sleep parameters

We recorded EEG, EMG and activity using the NeuroLogger® to determine sleep parameters. The protective cover was removed from the animals' head and the dummy replaced by the activated NeuroLogger® (sampling frequency 200 Hz). Subsequently, the protective cover was reconnected. This procedure took about 30 s and could be performed without sedating the animal. The animal received a piece of gummi arabicum or 0.5 ml Nutri-Cal (Albrecht, Aulendorf, Germany) as reward and was placed back in its homecage. All animals investigated in this study showed a quick habituation to the experimental manipulation. For the long-term recording experiment, NeuroLoggers® were changed daily (at varying times of the day) in



**Fig. 2.** Photograph of a marmoset pair implanted with NeuroLoggers®.





**Fig. 3.** Representative 24 h hypnogram showing the distribution of wake, NREM sleep and REM sleep of an individual marmoset monkey under baseline conditions. The white and black bar at the bottom indicates the light/dark-cycle, dim light is marked in grey.

order to download the data. For the SD experiment, the NeuroLogger<sup>®</sup> was removed 3 days after SD.

The recorded data were downloaded from the memory chip of the NeuroLogger<sup>®</sup> and saved in a hexadecimal format. They were then transformed to a text file using a Matlab routine (developed and kindly provided by Andrea Plano, University of Aberdeen) and imported into a sleep analysis program (SleepSign, Kissei America Inc., Irvine, CA, USA). The combined EEG, EMG and actimetry signals were classified in 10 s epochs as wakefulness, NREM sleep or REM sleep. Initial autoscoring was performed using SleepSign's waveform and logic algorithm. Each recording was then checked and, if necessary, corrected by an experienced observer.

Wakefulness was characterized by high-frequency and low voltage EEG activity, relatively high and often irregular EMG activity, and often a relatively high number of activity counts in the accelerometer recording. During NREM sleep, the EEG activity had high amplitude and was dominated by 1–4 Hz delta waves or sleep spindles, whereas EMG amplitude was low and no or very few accelerometer counts occurred. REM sleep was characterized by low-voltage EEG activity, combined with low or absent EMG and accelerometer signals. Based on this scoring, the time spent in each of the vigilance states, the number of episodes and the duration of the episodes were calculated for 30 min intervals and for the complete light and dark period. In addition to vigilance state analysis, the signals recorded during the sleep deprivation experiment were subjected to spectral analysis by fast Fourier transformation using SleepSign. As an indicator of sleep intensity, the EEG power in the 1–4 Hz delta range was calculated for all NREM sleep epochs. To correct for individual differences in the strength of the EEG-signal, delta power values were normalized by expressing them relative to each animal's own average 24 h baseline delta power (referred to as slow wave activity; SWA). The accumulation of NREM sleep delta power over time was calculated by multiplying NREM sleep SWA by NREM sleep time (referred to as cumulative slow wave energy; SWE).

#### 2.5.2. Locomotor activity

The recording of activity was performed by the accelerometer integrated in the NeuroLogger<sup>®</sup> in parallel to EEG and EMG recordings. The accelerometer counts were used to determine 24 h activity rhythms. The number of counts per 10 s interval was exported using SleepSign, and then averaged per minute. The long-term recording results were further processed into weekly 5 min intervals using a moving average method.

#### 2.5.3. Core body temperature

Core body temperature recording began directly after surgery and was carried out continuously until the end of the experiment. The implanted transmitter sent a temperature-dependent frequency-modulated signal to a receiver near the cage. Temperature was sampled and transmitted at 10 s intervals. The signals were transferred to a PC-based acquisition program (eDacq Version 1.7x, EMMS, Bordon, UK) and saved continuously from 6:20 to 6:00 the following day. The 20 min pause in recording allowed for saving and backing up the recorded data. For individual animals, data was averaged in 1 min bins within each day and, subsequently, averaged across 7 days to obtain weekly averages.

#### 2.6. Data analysis and statistics

Due to the small number of animals, statistical analysis was limited and only performed in the long-term recording experiment to assess within-animal changes in sleep parameters, locomotor activity, and core body temperature across the different recording periods. This was done to determine the stability of these parameters over time when recorded with the NeuroLogger<sup>®</sup>. For sleep parameters, the percentage of each vigilance state, the number of episodes and the duration of episodes for the 11.5 h dark phase were compared between the recording periods. For activity and temperature, we calculated and compared the area under the curve (AUC) (baseline set at 0), separately for the light phase (6:30–19:00, including dim light periods) and the dark phase (19:00–6:30), for the different recording periods.

Analyses were performed for each animal separately using the 7 daily values per recording period with repeated measures ANOVA for normally distributed data and nonparametric Friedman-ANOVA of repeated measures in case of not normally distributed data, followed by post hoc Student-Newman-Keuls test when appropriate. Differences between conditions were considered statistically significant at the 95% confidence level ( $p < 0.05$ ).

SigmaPlot 11 (Systat Software Inc., San Jose, CA, USA) was used to calculate the AUC of daily 1 min averages for locomotor activity and core body temperature

in the light and dark period separately. These values were statistically compared as described above. In addition, the interaction between activity and core body temperature was investigated using Spearman's rank correlation coefficient.

### 3. Results

#### 3.1. Long-term recording experiment

##### 3.1.1. Sleep-wake rhythm

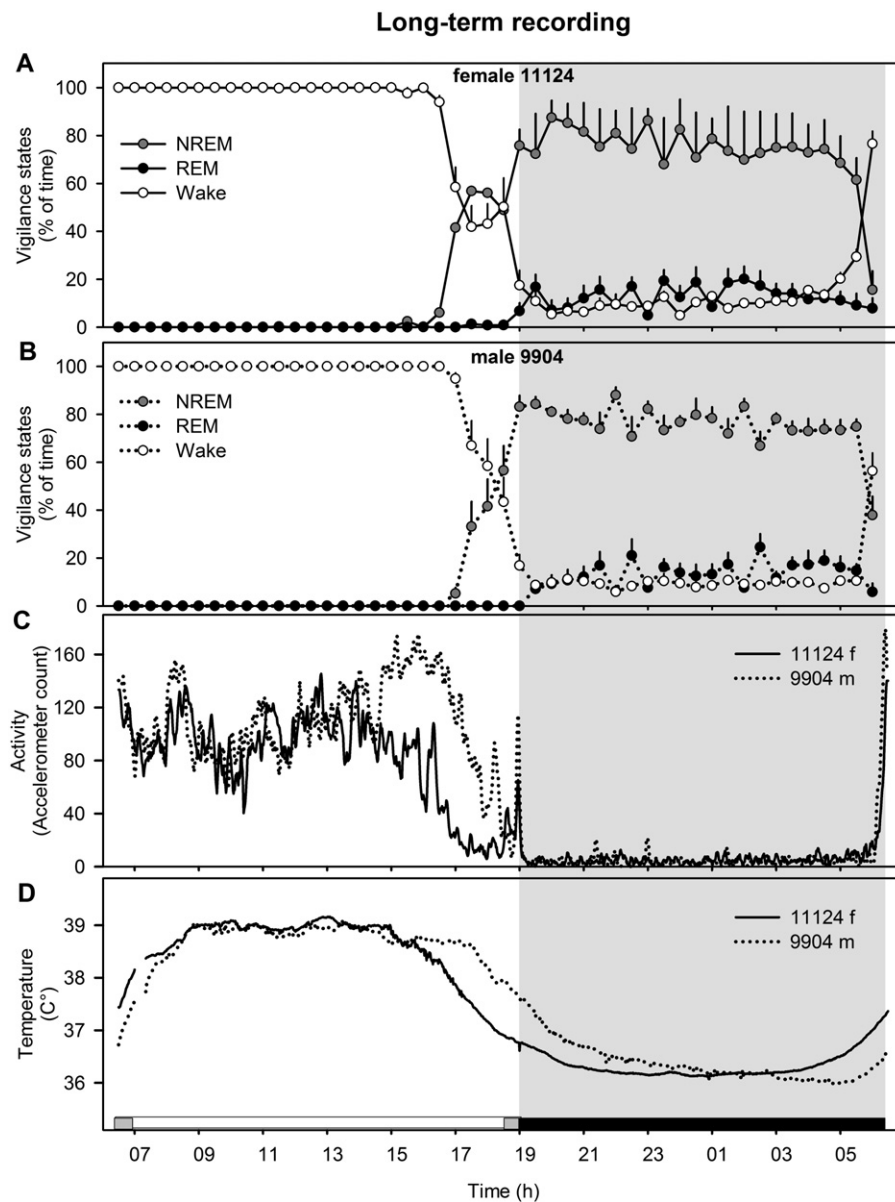
A representative 24 h hypnogram of a single-housed marmoset monkey is shown in Fig. 3. The average sleep-wake patterns from week 3 post-implantation of both animals investigated in this study are shown in Fig. 4A (female) and B (male). The animals spent most of the light phase awake. Approximately one hour before the end of the light-phase, wake duration decreased and short periods of sleep occurred; this was almost exclusively NREM sleep, as REM sleep rarely occurred during the light phase. The dark phase clearly represents the main rest period and was characterized by cyclic alternations of NREM and REM sleep ( $88.29 \pm 0.42\%$  sleep, consisting of  $75.37 \pm 0.49\%$  NREM and  $12.92 \pm 0.34\%$  REM sleep for the male and  $85.73 \pm 0.88\%$  sleep, consisting of  $73.00 \pm 0.59\%$  NREM and  $12.74 \pm 0.78\%$  REM sleep for the female).

About one hour before the end of the dark phase, the amount of sleep rapidly decreased until light phase percentages were reached again. In contrast to the male animal, which showed a largely monophasic sleep pattern throughout the experiment, the female showed short rest periods (mostly NREM sleep) around midday in some of the recording periods (data not shown). The results for percentage, number of episodes and episode duration for the different vigilance states in the dark phase of week 3 post-implantation are summarized in Table 1. Individual differences can be seen for all vigilance states. The male animal spent less time awake during the night, mainly due to a lower number of wake episodes. This was accompanied by an increase in NREM sleep percentage, which resulted from longer episode durations combined with decreased episode counts. While the total amount of REM sleep was comparable between the animals, the male animal had less REM sleep episodes of longer duration.

Within-animal comparison of the vigilance state parameters during the dark phase for the four 1-week recording periods proved that some of the parameters were stable, while others showed high variability over the course of the experiment. The results of the statistical analysis are summarized in Table 2.

Overall, the percentage of NREM sleep showed only small variations throughout the experiment. In the female marmoset, NREM percentage during the second week after implantation was significantly higher compared to all subsequent recording weeks. The significant decrease in episode number in weeks 3 and 9 and the parallel increase in episode duration led to stable percentages in these periods. A similar effect was observed in the male marmoset, since the decrease in episode duration in week 6 was accompanied by an increase in episode number. Compared to the female, the male marmoset had higher levels of NREM sleep with a small, but significant increase in weeks 6 and 9.

The percentage of REM sleep for the female animal showed a stepwise increase over time; the values from week 9 are



**Fig. 4.** Daily time course of sleep, activity and core body temperature in a female and a male marmoset monkey in the 3rd week after implantation (long-term recording experiment): (A) distribution of vigilance states in 30 min intervals for female 11124 (means of 7 recordings  $\pm$  SEM); (B) distribution of vigilance states in 30 min intervals for male 9904 (means of 7 recordings  $\pm$  SEM); (C) activity (means of 7 recordings, smoothed with 5 min moving average method); (D) core body temperature (means of 7 recordings, smoothed with 5 min moving average method). The white-and-black bar at the bottom indicates the light/dark-cycle, dim light is marked in grey.

significantly higher than in the other periods. The number of episodes differed between weeks 2 and 3 and the episodes were shorter in week 3 compared to weeks 6 and 9. In contrast, REM sleep in the male marmoset was highly stable over the course of the experiment. No obvious changes between the recording periods were observed.

The percentage of wake did not vary significantly in the female marmoset, but the number of episodes showed a trend to decrease throughout the experiment. A significant difference was found between the 3rd and 9th week post-implantation. This was accompanied by a significant increase in episode duration (weeks 2 and 3 vs. weeks 6 and 9). In the male marmoset monkey, we found a significantly lower percentage of wake in week 6 compared to week 3. This decrease resulted from a significantly lower episode number compared to the other recording periods. In contrast to the female marmoset, wake episode durations for the male were stable throughout the experiment.

### 3.1.2. Locomotor activity

The 24 h rhythm of locomotor activity of both animals in week 2 post-implantation is depicted in Fig. 4C. Activity was mainly restricted to the light phase. The female marmoset exhibited a gradual reduction in activity level in the last 4 h of the light phase. In contrast, the male remained active for 2 additional hours and then showed a steeper decline in activity during the last 2 h of the light phase. During the night, both animals showed no or minimal activity. The end of the rest phase was marked by a sudden increase in activity counts about 10 min before lights-on, which demonstrates that the animals became active before the lights turned on.

Fig. 5A and B show the AUC values for the light and dark phase of the different recording periods for the female and male marmoset, respectively. No significant differences in activity were found during the light phase for both animals as well as the dark phase for the female marmoset. The male showed a significantly higher AUC in the dark phase of the second week compared to all other periods.

**Table 1**

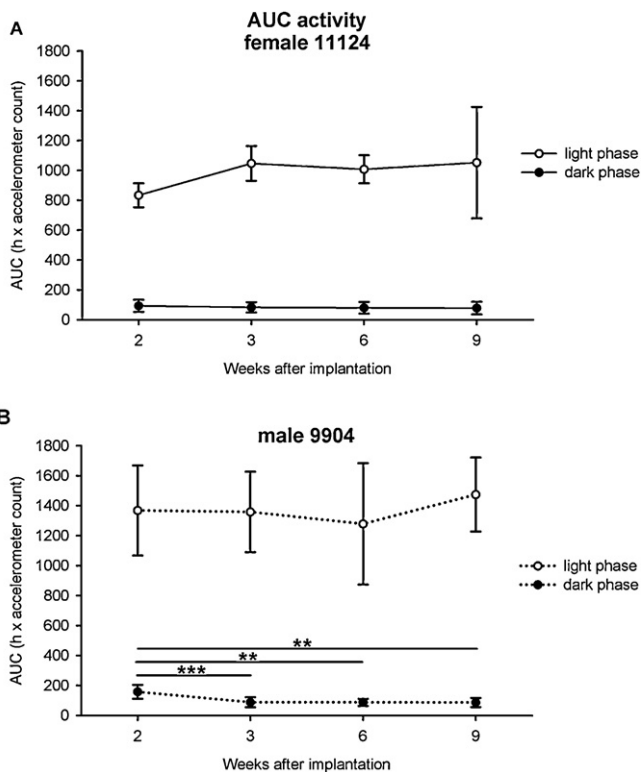
Amount, duration and episode length of vigilance states in the different recording periods of the long-term recording experiment.

Week after implantation		Wake	NREM	REM
% time				
2	Male	11.43 ± 0.54	75.53 ± 0.54	13.04 ± 0.34
	Female	12.94 ± 0.55	74.97 ± 0.43	19.09 ± 0.27
3	Male	11.71 ± 0.42	75.37 ± 0.49	12.92 ± 0.34
	Female	14.27 ± 0.88	73.00 ± 0.59	12.74 ± 0.78
6	Male	9.37 ± 0.48	77.97 ± 0.46	12.67 ± 0.25
	Female	14.19 ± 0.49	71.90 ± 0.33	13.91 ± 0.53
9	Male	10.42 ± 0.36	77.49 ± 0.59	12.09 ± 0.31
	Female	12.43 ± 0.67	71.99 ± 0.81	15.58 ± 0.54
Number of episodes				
2	Male	91.14 ± 3.99	99.14 ± 4.14	15.43 ± 0.81
	Female	119.57 ± 4.9	127.14 ± 4.54	19.00 ± 0.79
3	Male	89.86 ± 2.23	96.29 ± 2.78	15.43 ± 0.53
	Female	129.29 ± 5.71	140.00 ± 4.72	24.86 ± 1.72
6	Male	73.43 ± 2.23	85.71 ± 2.04	16.29 ± 1.15
	Female	107.00 ± 8.21	114.86 ± 6.7	20.29 ± 1.25
9	Male	86.29 ± 3.56	95.00 ± 3.45	14.71 ± 0.96
	Female	95.00 ± 4.36	104.00 ± 3.73	21.14 ± 0.96
Average episode duration (s)				
2	Male	51.71 ± 3.99	318.00 ± 12.15	354.43 ± 17.91
	Female	44.71 ± 4.9	245.86 ± 10.42	267.00 ± 15.8
3	Male	53.71 ± 2.23	325.14 ± 9.03	349.14 ± 15.44
	Female	45.29 ± 5.71	217.00 ± 8.24	218.57 ± 21.58
6	Male	52.71 ± 2.46	377.29 ± 9.59	331.00 ± 22.4
	Female	55.57 ± 8.21	263.86 ± 14.83	288.29 ± 16.03
9	Male	49.86 ± 3.29	339.43 ± 11.11	346.14 ± 20.64
	Female	54.00 ± 4.36	288.71 ± 11.59	307.00 ± 12.10

**Table 2**

Statistical differences of vigilance state parameters in the dark phase for a female and male marmoset monkey as determined by repeated measures ANOVA for normally distributed data and Friedman repeated measures ANOVA on ranks for not normally distributed data (number of wake episodes for the male marmoset) with Student–Newman–Keuls post hoc test; n.s. = not significant.

Comparison		Wake	NREM	REM
% time				
Week 2 vs. 3	Male	n.s.	n.s.	n.s.
	Female	n.s.	0.016	n.s.
Week 2 vs. 6	Male	0.018	0.01	n.s.
	Female	n.s.	0.002	n.s.
Week 2 vs. 9	Male	n.s.	0.016	n.s.
	Female	n.s.	<0.001	<0.001
Week 3 vs. 6	Male	0.013	0.012	n.s.
	Female	n.s.	n.s.	n.s.
Week 3 vs. 9	Male	n.s.	0.025	n.s.
	Female	n.s.	n.s.	0.001
Week 6 vs. 9	Male	n.s.	n.s.	n.s.
	Female	n.s.	n.s.	0.011
Number of episodes				
Week 2 vs. 3	Male	n.s.	n.s.	n.s.
	Female	n.s.	n.s.	0.022
Week 2 vs. 6	Male	$p < 0.05$	0.028	n.s.
	Female	n.s.	n.s.	n.s.
Week 2 vs. 9	Male	n.s.	n.s.	n.s.
	Female	n.s.	0.032	n.s.
Week 3 vs. 6	Male	$p < 0.05$	n.s.	n.s.
	Female	n.s.	0.011	n.s.
Week 3 vs. 9	Male	n.s.	n.s.	n.s.
	Female	0.011	0.002	n.s.
Week 6 vs. 9	Male	$p < 0.05$	n.s.	n.s.
	Female	n.s.	n.s.	n.s.
Average episode duration (s)				
Week 2 vs. 3	Male	n.s.	n.s.	n.s.
	Female	n.s.	n.s.	n.s.
Week 2 vs. 6	Male	n.s.	0.003	n.s.
	Female	0.025	n.s.	n.s.
Week 2 vs. 9	Male	n.s.	n.s.	n.s.
	Female	0.017	n.s.	n.s.
Week 3 vs. 6	Male	n.s.	0.005	n.s.
	Female	0.02	0.03	0.033
Week 3 vs. 9	Male	n.s.	n.s.	n.s.
	Female	0.017	0.007	0.008
Week 6 vs. 9	Male	n.s.	0.016	n.s.
	Female	n.s.	n.s.	n.s.

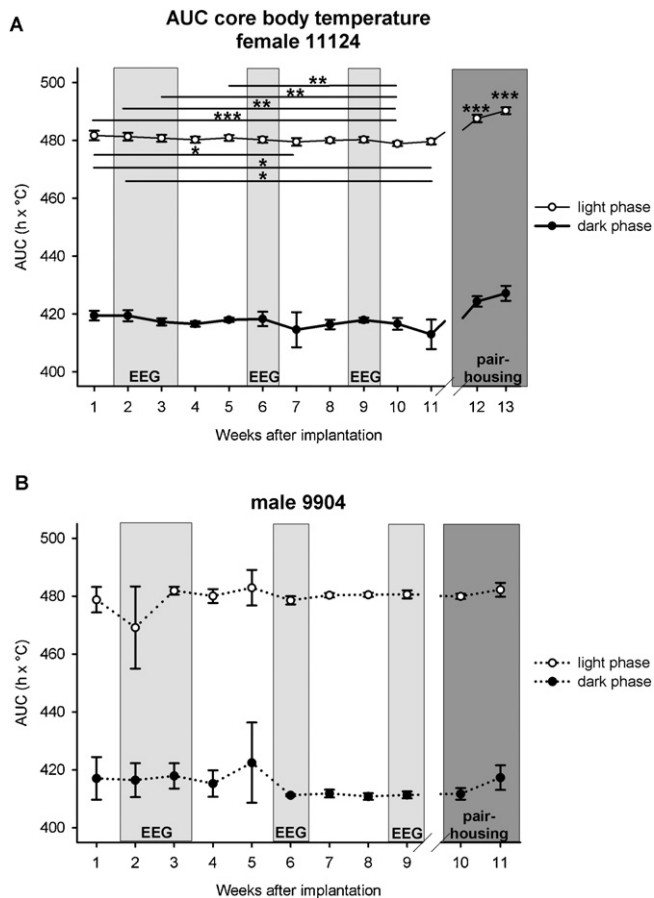


**Fig. 5.** Area under the curve (AUC) values of activity for (A) female and (B) male marmoset monkey in the light and dark phase of the 2nd, 3rd, 6th and 9th week after implantation. All data are expressed as means of 7 recordings ± SD. Horizontal bars with asterisks indicating significant differences between recording weeks (\*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

### 3.1.3. Core body temperature

The marmosets displayed a pronounced daily rhythm in core body temperature, with a difference between light and dark period of about 3 °C (Fig. 4D). In the first 2 h after lights-on, body temperature increases gradually to a stable daytime level of approximately 39 °C. The body temperature of the female marmoset began to decrease 4 h prior to dark onset. This trend continued in the first hour of the dark period until a stable nighttime temperature of around 36 °C was reached. In parallel to the differences in activity patterns between the male and the female, the decrease in the male's body temperature was shifted; it started to decline 2 h later and continued for the first 2 h of the dark period until it reached the stable nighttime level. In the last 1–1.5 h of the dark phase, the body temperature of both animals started to rise again, in parallel to the increase in wakefulness and at least partly preceding the onset of activity.

The body temperature curves for the different weeks showed only small changes throughout the experiment (data not shown). This was also reflected in the AUC values (Fig. 6). In the female animal, AUC values showed slight, but significant variations during the light phase under single-housed conditions (Fig. 6A). The highest AUC value was recorded in the first week after implantation; the AUC values in subsequent weeks showed a decreasing course. The observed significant differences between specific recording



**Fig. 6.** Area under the curve (AUC) values of core body temperature of a (A) female and (B) male marmoset monkey in the light and dark phase in single- and pair-housing conditions. Light-grey boxes indicate periods with simultaneous EEG recording. Data are expressed as means of 7 recordings  $\pm$  SD. Horizontal bars with asterisks indicating significant differences between recording weeks (\* $p < 0.1$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

weeks were not related to the NeuroLogger® recording weeks. In the dark phase, AUC values for the female animal in single-housed conditions were not significantly different. The AUC values for the male animal in single-housed conditions did not show significant differences in the light or dark phase (Fig. 6B). Therefore, the experimental manipulation of changing the NeuroLoggers® had no major influence on core body temperature.

Pair-housing lead to a phase-shift in the decrease in body temperature at the end of the light (and beginning of the dark) phase for the female; this resulted in the synchronisation of the temperature curves of both animals. This shift was also reflected by an increase in the AUC values of the female, both in the light and dark phase. However, these changes were only significant during the light phase. In contrast to the female, no changes in the body temperature rhythm and AUC values occurred in the male.

## 3.2. Sleep deprivation experiment

### 3.2.1. Baseline sleep

The baseline recordings for the sleep deprivation experiment were performed in pair-housed conditions. The mean 24 h rhythm of the sleep-wake cycle and the distribution of vigilance states (Fig. 7) are comparable to those observed in single-housed conditions for the long-term recording experiment. Both animals spent a large part of the dark phase asleep. NREM sleep EEG slow wave activity as an indicator of sleep intensity was highest in the first half

of the night and gradually decreased over the course of the night. When NREM occurred in the form of naps during the light phase, then slow wave activity was usually below 100% of average.

The 24 h rhythm of locomotor activity was comparable to that observed in single-housed conditions for the long-term recording experiment.

### 3.2.2. Sleep deprivation and subsequent recovery period

During the 6 h sleep deprivation, the animals were completely deprived of REM sleep and almost fully deprived of NREM sleep (Fig. 7). Relative to the same baseline period, 6 h sleep deprivation resulted in a mean loss of 244.9 min NREM and 49.1 min REM sleep. During the remainder of the dark phase following sleep deprivation, animals showed an increase in NREM sleep SWA, suggesting an increase in sleep intensity to compensate for the sleep that was lost. The peak in NREM SWA was 144.8% of baseline activity and therefore about 15% higher than peak baseline SWA. Also, the amount of NREM sleep was somewhat elevated, particularly during the first couple of hours of the subsequent light phase. We calculated the SWE of NREM, sleep since the marmosets appeared to compensate for NREM sleep loss during SD by increasing both NREM sleep time and intensity. Even though the incline of the SWE curve after sleep deprivation was steeper, the cumulative SWE remained below baseline levels until the end of the light phase.

REM sleep for the remaining dark phase was slightly decreased compared to the same baseline period (48.8 min compared to 50.4 min), mainly resulting from a reduction in REM sleep time during the first 3.5 h following SD, while an increase above baseline levels occurred in the last 2 h.

Locomotor activity was also affected by sleep deprivation. During SD itself, the animals showed more locomotion, but activity stayed below daytime values. During the light-phase following SD, overall activity was reduced in many of the 30 min intervals.

### 3.2.3. Second and third recovery day

Although the animals increased NREM sleep time and intensity after 6 h SD, these changes did not fully make up for the sleep loss. To investigate whether there was a delayed or prolonged effect of 6 h SD, we analysed the second and third full recovery day (Fig. 8).

On the second recovery day, we observed only small changes in the vigilance states compared to baseline. Total NREM sleep duration was slightly increased by 10.7 min during the dark phase and 20.0 min during the light phase. Again, NREM sleep SWA was elevated compared to baseline, especially in the first half of the dark period with a peak of 144.7% of baseline average. On the second recovery day, the cumulative NREM SWE was constantly elevated above baseline levels.

In contrast, REM sleep rebound was delayed and most pronounced on the second recovery day with an increase of total REM sleep duration by 15.7 min.

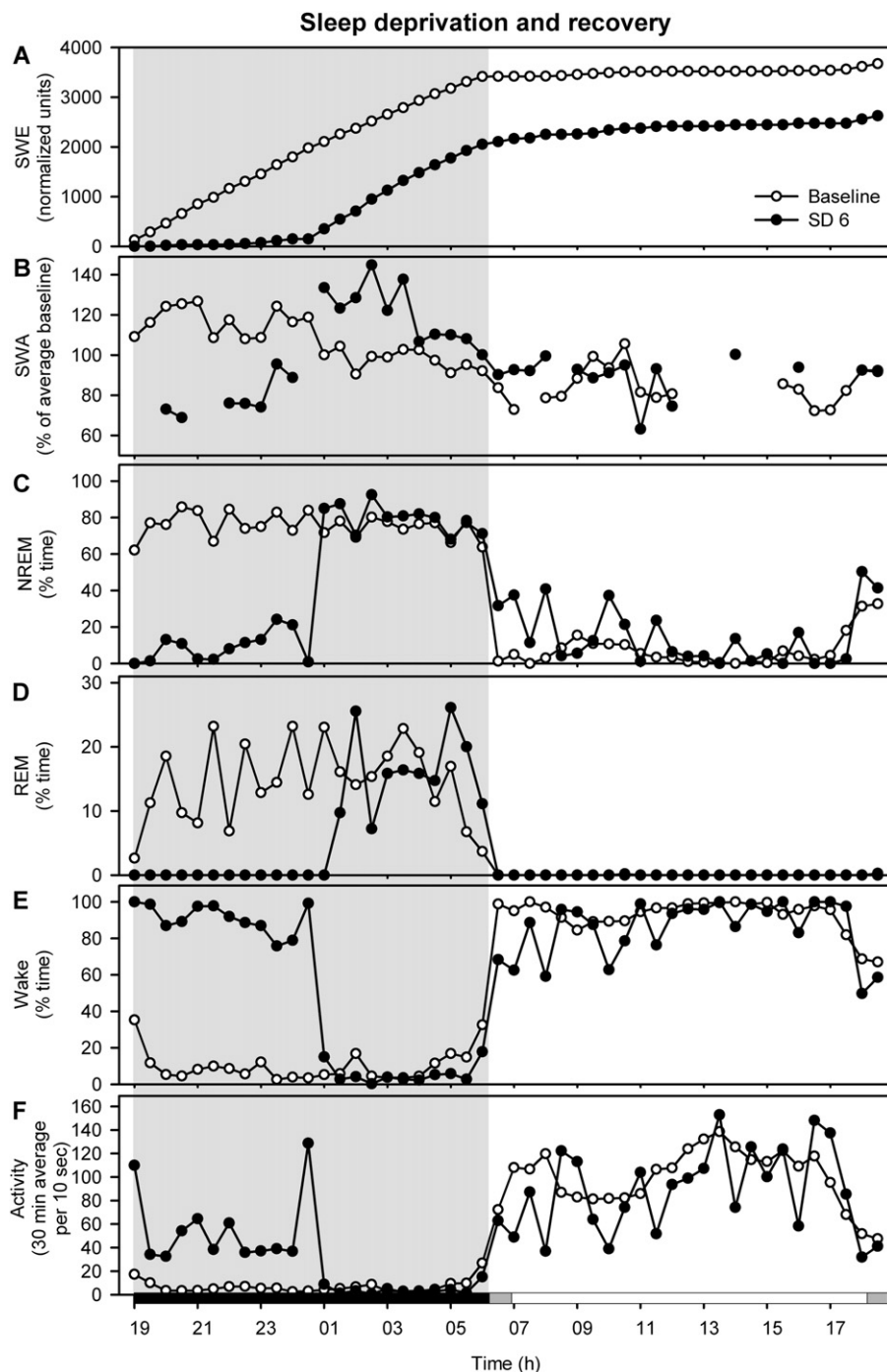
The effects on locomotor activity were small; changes occurred mainly in the second half of the light period. Activity was reduced during this part of the day, which was most likely due to a greater number of naps.

On the third recovery day, no obvious changes in sleep and activity pattern of the animals could be seen compared to baseline.

## 4. Discussion

In this study, we investigated the remote recording of full 24 h rhythms of sleep, locomotor activity and core body temperature in non-restrained marmoset monkeys. We were able to show that after some modifications, the recording devices employed were suitable in different experimental settings, such as long-term





**Fig. 7.** Changes in sleep architecture, sleep EEG and activity induced by 6 h sleep deprivation (SD) during the dark phase. Data in all graphs are means of both animals for the second experimental SD. Refer to the text for further details. The black-and-white bar at the bottom indicates the dark/light-cycle, dim light is marked in grey.

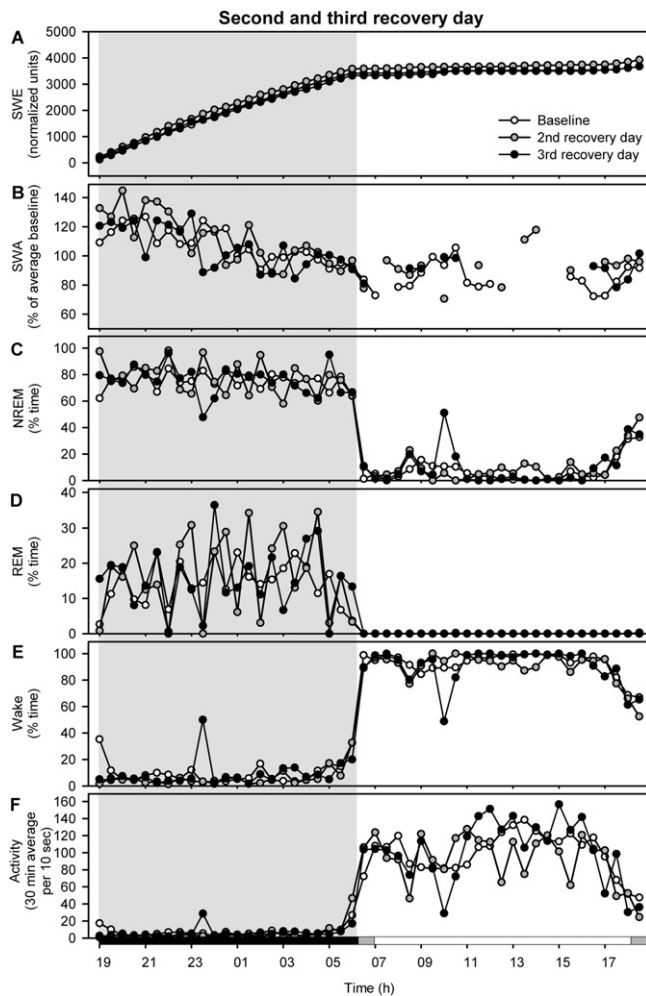
registrations in single-housed conditions, pair-housed conditions and during a sleep deprivation experiment.

#### 4.1. Methodology

We adapted the NeuroLogger® system for the recording of EEG, EMG and activity counts by an integrated movement sensor to assess sleep parameters in the marmoset. During the long-term recording experiment, the NeuroLoggers® were changed daily during the light phase for downloading of the data, which resulted in about 30 min missing data. To overcome this problem, we extended the recording time and optimized the recording protocol for the

subsequent SD experiment. The NeuroLoggers® were connected before SD and left unchanged for the next 3 days, which was the approximate maximum recording capacity and battery life.

The accelerometer integrated in the NeuroLogger® allowed for continuous activity recordings. However, due to the fixation on the head of the animal, we cannot exclude that slight head movements were also counted as activity in addition to general locomotor activity. We do not think that this contributed substantially to the overall interpretation of the activity data, but comparison with another activity measurement and video recordings may be required to accurately ascertain the exact contribution of head movements to the activity count of the accelerometer.



**Fig. 8.** Sleep architecture, sleep EEG and activity on the second and third recovery day. Data in all graphs are means of both animals after the second experimental sleep deprivation. Refer to the text for further details. The black-and-white bar at the bottom indicates the dark/light-cycle, dim light is marked in grey.

## 4.2. Baseline recordings

### 4.2.1. Sleep parameters

In the present study, marmosets showed a more or less monophasic sleep pattern with more than 85% sleep occurring during the dark phase, but occasional daytime naps consisting primarily of NREM sleep were also observed. The marmosets displayed a somewhat phase-advanced sleep-wake rhythm relative to the LD cycle, with a gradual increase in sleep starting about 2 h before the onset of the dark phase and a gradual increase in wakefulness about 30 min before the onset of the light phase. This contrasts with the results reported by Crofts et al. [38], who observed that the animals usually fell asleep after dark onset and awoke at the beginning of the light phase. However, our results agree with the findings of the chronobiological studies of locomotor activity in marmosets performed by Erkert et al. [32,36,49]. These studies showed that the free running circadian rhythm of marmosets is shorter than 24 h, which translates to a phase-advanced rhythm.

We found that the daytime sleep that occurred in our marmoset monkeys before the onset of the dark phase mostly consisted of NREM sleep. These NREM sleep episodes were of rather short duration as compared to NREM sleep during the night. This may explain the lack of daytime REM sleep. The high fragmentation and, consequently, premature termination of NREM episodes might prevent the animals from entering REM sleep.

While the percentage of vigilance state parameters during the night phase agrees with earlier reported values [38,39,41], we found higher numbers of wake episodes in both animals and more REM episodes in the female. These differences most likely result from different scoring techniques. By using a stage length of 10 s compared to 30 s used in other studies, our scoring was more sensitive to changes in vigilance states and most likely resulted in higher episode counts.

Comparison of recording periods in the long-term recording experiment showed a high variability in some parameters, particularly for the female investigated in this study, while other parameters were remarkably stable. Notably, we found that the overall percentages of the sleep stages were characterized by low variations over time, indicating a strong homeostatic regulation, while the episode numbers showed a decreasing trend over the course of the experiment. A possible factor that could have an influence on sleep architecture is the short daily disruption for changing of the NeuroLoggers®.

We know from other species like rats, that experimental manipulation can lead to changes in sleep-wake rhythms and that this influence can be reduced by handling the animals prior to experiments [50]. Even though we can conclude from the temperature recording results that the animals were well adapted to the experimental procedure, it is possible that the observed changes reflect a consolidation of sleep due to a continuous adaptation process over the course of the experiment.

Ehlers and colleagues showed that group housing in rats, which is associated with an increased well-being of the animals, reduced vigilance state changes [51]. We did not find a reduction in state changes in pair housing conditions for the marmosets. The baseline recordings for the pair-housed animals in the SD experiment showed similar episode counts compared to the single-housed animals in the long-term recording experiment.

In addition, the possibility that the observed changes reflect spontaneous random variations has to be taken into account. Also, we cannot completely exclude the influence of external factors, such as other experiments that were performed concurrently in the animal facility.

Due to the small sample size in this study, we also cannot say if the differences observed between the animals reflect individual physiological variations or if there is also a larger effect of age and gender. Future studies with more animals will help to clarify this issue.

### 4.2.2. Locomotor activity

The activity patterns of marmosets are reported to be highly variable. While bimodal patterns are predominant in animals in the wild, uni- and multimodal patterns are observable in animals in captivity [32]. In this study, we found that activity was largely restricted to the light phase, as also previously reported in the literature [32,36,37,49], except for an early onset of activity about 10–20 min before lights on.

The male marmoset recorded in this study exhibited increased activity during the adaptation period in the dark phase. It is known from studies in rats that experimental manipulations can lead to increased activity [52]. Therefore, we assume that the increased activity at the beginning of the experiment resulted from unfamiliar handling and that the reduction in the subsequent weeks was a result of habituation.

### 4.2.3. Core body temperature

Marmoset monkeys display a clear diurnal rhythm in core body temperature, with highest temperatures during the light phase and lowest temperatures during the dark phase. The differences in temperature that we observed between the light and dark phase are consistent with previous studies [33,49,53]. The male marmoset

showed a later temperature decline at the end of the light phase than the female, which is in agreement with the results of the sleep and activity registrations.

The comparison of recording weeks did not show a significant difference in body temperature levels in weeks with and without NeuroLogger® recordings. This leads us to the conclusion that the animals were well adapted to the changing procedure and no stress-related differences in body temperature levels occurred. However, we did observe some significant changes in the nighttime body temperature levels of the female marmoset that occurred in a regular 4 week interval. It is known from humans that core body temperature varies with different phases of the estrous cycle as a result of changes in progesterone and estradiol levels; females have higher core body temperatures in the luteal phase compared to the follicular phase. While progesterone cause an increase, estradiol results in a decrease in body temperature [54–56]. Female marmosets have a hormone profile that is comparable to humans, with a 28–30 day ovarian cycle and a follicular phase of about 8 days. [28]. Therefore, it is highly likely that the observed variation in core body temperature was a result of variations in hormone levels. Future studies measuring fluctuations in female hormone levels as a correlate of core body temperature could confirm this hypothesis.

Pair housing led to the synchronisation of temperature rhythms between the two marmosets. We also found that the change in housing conditions led to an elevation of core body temperature in the female marmoset. This might be a result of increased overall activity. Also, social conflicts that lead to the establishment of a stable rank order could increase body temperature [57,58]. To minimise the risk of social conflicts by the change in housing conditions, the animals were kept in neighbouring cages separated by a grid to allow visual, acoustic and olfactory communication between the animals and avoid isolation when single housing was necessary due to the experimental setup. However, since the animals were reunited after a period of 11 weeks, this also should be taken into account, even though no signs of conflicts were observed.

#### 4.3. Sleep deprivation and recovery

As expected, SD was followed by a clear sleep rebound, which partly compensated for the sleep that had been lost. First of all, a NREM sleep rebound occurred, which was reflected by an increase in NREM sleep time and NREM sleep intensity. NREM sleep SWA in the subsequent recovery and the first recovery night was around 15% above the peak baseline levels.

REM sleep rebound was delayed and occurred most pronouncedly in the second recovery night.

However, the increase did not fully compensate for the REM sleep that had been lost during SD. The priority for NREM sleep recovery over REM sleep recovery has also been reported in humans. In other words, a moderate extension of waking time is not always followed by increased REM sleep time [5,7,12]. Similarly, in established laboratory species such as the rat, only a mild or NREM sleep rebound was observed following acute SD procedures [4,6], while prominent REM rebounds that did not fully make up for the lost sleep occurred in prolonged SD of more than 24 h [3,59].

#### 4.4. Conclusions

In conclusion, we were able to show that the use of the NeuroLogger® for sleep and activity recordings in combination with telemetric Remo200 transmitters enabled remote long-term recordings of diurnal rhythms of freely moving marmosets in different housing conditions and experimental settings. Marmosets are day-active animals with a more or less monophasic sleep pattern. Rhythms of sleep, activity and body temperature are more similar to humans than that of widely used laboratory species such as rats

and mice. This, and the fact that the marmoset is a well-established model for neurobiological studies make it an interesting species for sleep and circadian rhythm studies with broad applications.

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